88 A novel approach to the development of a murine model of cystic fibrosis associated chronic pulmonary bacterial infection

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Research into the immunopathogensis of chronic bacterial colonisation of cystic fibrosis (CF) patients has been hampered by lack of a suitable animal model. Whilst models of chronic infection have been described, they rely on the use of artificial embedding material to prevent rapid clearance of the bacteria. We hypothesised that careful selection of the bacterial isolate, mouse strain and infection regime, will result in a clinically relevant murine model of chronic *S. aureus* (SA) and *P. aeruginosa* (PA) infection without the need for embedding materials. Previously published models have largely used PA01 and lab attenuated SA strains. We have screened several hundred clinical isolates of SA and PA and selected 5 strains of each for murine infection experiments, based on genotype and phenotype. The selected SA isolates include haemolytic strains from CF and non-CF sputum. The PA isolates originated from CF or ICU patients and were selected based on phenotype including ability to form biofilm *in vitro*. Significant differences in the *in vivo* virulence of these strains have been shown. Equally essential is mouse strain. C57BL6 mice are used almost exclusively, however this strain are inherently resistant to bacterial infection. We have compared the survival and colonisation of C57BL6, A/J, BALBc, Biozzi, FVB/N, NIH, SJL, CD1, MF1 and NMRI female mice with PA and SA. Identifying a combination of mouse and bacterial strain resulting in the prolonged survival of the animal whilst maintaining chronic pulmonary infection, will allow investigation of the intricate immunological and physiological disease processes involved during chronic pulmonary infection of CF patients.

89 Identical obligate anaerobic bacteria in sputum of patients with COPD and CF

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In sputum of CF patients next to the aerobic bacteria also obligate anaerobic bacteria exist. Similarly as in CF, patients with Chronic Obstructive Pulmonary Disease (COPD) suffer from chronic infection and inflammation and produce large amounts of sputum. Therefore, we investigated if also in the lungs of COPD patients obligate anaerobes exist. 13 patients (5 female, 8 male, age 53–86 yrs, mean 69.8±10.3 yrs) with COPD GOLD standard stage IV spontaneously expectorated one or more sputum samples. Bacteria were identified using the Crystal® (Becton Dickinson, Heidelberg, Germany), the Vitek® (BioMérieux, Marcy L'Etoile, France) and the Rapid AnaII® (remel, Lenexa, KS, USA) identification systems. Bacteria were quantified using 10-fold dilutions, five different agar media, and an anaerobic bench. Oxygen partial pressure (pO_2) was measured in 4 sputum samples using an oxygen electrode (MI-730, Microelectrodes Inc., Bedford, NH, U.S.A.). In all COPD patients, at least one (maximum three) obligate anaerobic strain was identified. The most frequently occurring species were *Staphylococcus saccharolyticus* (11×), *Peptostreptococcus prevotii* (6×), *Ps. anaerobius* (3×), and *Veillonella* spp. (2×). *Ps. asaccharolyticus*, *Clostridium difficile*, and *C. histolyticum* were found once each. All of these species have also been detected in CF sputum before. Bacterial numbers ranged from 8×10^4 cfu/ml to 8×10^7 fu/ml. pO_2 was slightly reduced to 16.7±2.5%. Compared to CF patients, the reduction of the pO_2 in sputum of our COPD patients is low. However, the presence of the same obligate anaerobic genera and species in COPD and CF sputum stands in favour of a shared source of the bacteria.

90 Investigation of immunogenic outer membrane proteins of *Burkholderia cepacia* complex using serum from cystic fibrosis patients

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Burkholderia multivorans and *B. cenocepacia* are the two most frequently acquired species of *Burkholderia cepacia* complex (Bcc) in CF patients. This pathogen is transmissible and very difficult treat due to its antimicrobial resistance.

Objective: To identify the immunogenic outer membrane proteins (OMP) of *B. multivorans* and *B. cenocepacia* using CF patient serum in order to aid the design of prophylactic therapies.

Methods: The OMPs were isolated individually from two *B. cenocepacia* strains and two *B. multivorans* strains, separated by 2-D electrophoresis and blotted onto PVDF membranes. Immunogenic OMPs were detected by probing blots with serum from CF patients colonised with Bcc followed by chemiluminescent detection. Proteins spots were matched to corresponding spots on a Coomassie blue stained gel, excised and subsequently identified by MALDI-TOF/MS.

Conclusions: Among the 140 immunogenic proteins identified from the four Bcc strains, six of them were common to all strains, twelve were identified in both *B. multivorans* strains and seven of them were identified in both *B. cenocepacia* strains. The OMP Porin, OprM and chaperonin GroEl were all identified as being considerably immunogenic in all strains.

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91 Host neutrophil interactions with biofilms of the CF pathogen, *Burkholderia cepacia* complex (Bcc)

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Burkholderia cepacia complex (Bcc) members are associated with patient mortality in CF as they may induce '*cepacia* syndrome', an acute deterioration of lung function with associated septicaemia. Most Bcc species are multi-drug-resistant and form biofilms, further reducing their antibiotic susceptibility. To ascertain whether Bcc biofilm formation confers advantages to the pathogen by evading host cell responses, we examined the interaction of neutrophils with Bcc biofilms *in vitro*. Confocal microscopy demonstrated that biofilm formation protected Bcc species from phagocytosis, illustrating that differentiated, neutrophil-like HL60 cells (dHL60s) remain at the exterior surface of Bcc biofilms >2h post-inoculation, impeding phagocytosis. Planktonic Bcc bacteria did form significantly less biofilm, up to 72h after inoculation, when initially inoculated in tandem with dHL60s ($p < 0.05$); however when added to biofilms already established for 24–72h, dHL60s lead to an increase in biofilm density. Neutrophils undergo apoptosis and disintegrate shortly after recruitment to the lung and repeat experiments demonstrated that whole-cell lysates of dHL60 also increased the density of established biofilms (48h). Interestingly, dHL60s secreted significant quantities of IL-8 <1h after contact with Bcc biofilm ($p < 0.05$), but degranulated less when added to older biofilms (48–72h). Hence, biofilms facilitate Bcc species persistence in the CF lung and the presence of neutrophils reinforces that biofilm. Therefore, strategies to improve neutrophil efficacy in clearing colonising Bcc must focus on overcoming biofilm-mediated resistance.

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